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INTERSPECIFIC CROSS BETWEEN DURUM WHEAT AND *AEGILOPS GENICULATA* TO TRANSFER RESISTANCE TO HESSIAN FLY (*MAYETIOLA DESTRUCTOR SAY.*).

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ABSTRACT. *Interspecific cross between Durum Wheat and Aegilops geniculata to transfer resistance to Hessian fly (Mayetiola destructor Say.).* Interspecific crosses between durum wheat (*Triticum durum*) and accessions of *Aegilops geniculata* were initiated the first year of the present program. Only those accessions that were resistant to Hessian fly (*Mayetiola destructor Say.*) were used with the objective to transfer the resistance to wheat. Embryo rescue of immature hybrid seeds was necessary. Two hybrids between *T. durum* and *A. geniculata* were produced and planted in the field the second year. They presented intermediate traits between their two parents and produced a progeny after selfing or backcrossing. Meiotic analysis of the pollen mother cells showed low pairing between parental chromosomes in the hybrids.

Key words. *Triticum durum*, *Aegilops geniculata*, interspecific cross, embryo rescue, mitotic and meiotic analyses.

RÉSUMÉ. *Croisement interspécifique entre le blé dur et Aegilops geniculata pour le transfert de la résistance à la mouche de Hesse (Mayetiola destructor Say.)* Des croisements interspécifiques entre le blé dur (*Triticum durum*) et des accessions d'*Aegilops geniculata* ont été initiés la 1ère année de ce programme. Seuls les accessions résistantes à la mouche de Hesse (*Mayetiola destructor* Say.) ont été utilisées dans l'objetif de transférer la résistance au blé. Le sauvetage d'embryons hybrides immatures a été nécessaire. Deux hybrides entre *Triticum durum* et *Aegilops geniculata* ont été produits et transférés au champs la 2ème année. Ils ont présenté une morphologie intermédiaire entre leurs deux parents et ont produits une descendance après autofécondation ou rétrocroisement. L'analyse méiotique des cellules mère du pollen a montré un faible appariement entre les chromosomes chez les deux hybrides.

Mots clés. *Triticum durum*, *Aegilops geniculata*, interspécifique croisement, sauvetage d'embryons, mitotic et méiotique analyses.

INTRODUCTION

Wheat yield improvement is dependent upon genetic diversity available in its genetic pool. This diversity has been subjected to high genetic erosion as a result of selection pressure (Porceddu *et al.*, 1988). Thus recourse to the genetic resources available in alien species has become more necessary in recent years (Sharma, 1995). The introduction of alien

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genetic material through interspecific or intergeneric hybridization allowed the transfer of genes for resistance to many diseases and pests (Dvorak, 1977; Jones *et al.*, 1995; Friebe *et al.*, 1996; Valkoun *et al.*, 1990). Even though, this method is confronted with incompatibility barriers that limit hybrid production. Several techniques are available to overcome these barriers, and affect gene introgression into wheat genome (Sears, 1981; Feldman, 1983 ; Baum *et al.*, 1992; Sharma, 1995; Mujeeb-Kazi, 1993). *A. geniculata* is a useful source of variation for wheat improvement, but very much unutilized. Very few hybridizations have been made of this species with durum wheat (Kimber & Abubaker, 1979; Sharma & Gill, 1983; Simeone & Blanco, 1985; Nasyrov & Ibraginova, 1982; Farooq *et al.*, 1990). In Morocco, durum wheat is grown widely but most cultivars are susceptible to Hessian fly. During some seasons, yield loss can reach up to 80% (Lhaloui *et al.*, 1992). Therefore, looking for new sources of resistance to this insect in alien species constituted the primary objective in the Moroccan national breeding program. Since then, many resistant accessions were selected in *Aegilops* species. Our goal is to transfer the Hessian fly resistance genes into Moroccan durum wheat and to explore the alien germplasm of wheat mainly for resistance to Hessian fly.

The objective of this paper is to report the result on the interspecific hybridization over three years and the characterization of hybrids produced between durum wheat and an *Aegilops geniculata* accession resistant to Hessian fly.

MATERIALS AND METHODS

Plant material used in this essay included local Moroccan varieties of durum wheat (Oum Rbia, Marzak, Cocorit and Tensift) and *A. geniculata* accessions (400150, 400151, 400159, 400161, 400163). *Aegilops* accessions were chosen because of their resistance to Hessian fly (El Bouhssini *et al.*, 1997). Field planting of wheat varieties was scheduled at different dates to enhance the chances of nicking with *Aegilops*. Direct and reciprocal crosses were made manually by spreading pollen on emasculated flowers. Two weeks later, immature hybrid seeds were taken from the mother plants, washed in 70° alcohol and then surface sterilized in 5% sodium hypochlorite solution. After a few washes in sterile distilled water, embryos were isolated from the seed and cultured on a modified MS medium (Sharma & Baenziger, 1986). When developed into plants, hybrids were treated with a solution containing 1% colchicine and 2% DMSO, for 5 hours. After two to three weeks acclimatization under controled temperature and humidity, hybrids were planted in the field for their characterization. Plant, spike and leaf traits, fertility and resistance to some common diseases were scored.

Chromosome counts of both parents and hybrids were done on root tips as described by Endo and Gill (1984). Pollen mother cells from hybrids were analyzed for chromosome pairing. For this purpose, spikes were fixed in a 3:1 ethanol: acetic acid solution and anthers were squashed in 1% acetocarmine solution. Fifty

Parents crossed	Male parent	No of florets	% seed set	% rescuing	% plant formed
T. durum	*A. geniculata*	540	3.9	42.8	1.7
A. geniculata	*T. durum*	80	6.2	40	2.5

Table 1: Seed set and hybrid plants produced in *T. durum* x *A. geniculata* cross during the first year.

	% Seed set	% Plant formed	% Plant transferred to field conditions
Year 1	3.9	1.7	0
Year 2	5.0	1.8	10
Year 3	7.3	1.8	17,7

Table 2. Comparison of hybrid seed set in (*T. durum* x *A. geniculata*) cross over three years.

percent of the hybrid spikes were backcrossed to wheat parent and the others were left for selfing.

RESULTS AND DISCUSSION

Over 500 durum wheat flowers were emasculated and then pollinated with *Aegilops geniculata* pollen. Average seed set was lower in direct cross with wheat as the female than in the reciprocal one (tab. 1). However, normal embryos were more frequent in direct cross. Of the 15 embryos dissected only one was abnormal, while only 2 out of 5 embryos were normal in reciprocal cross. Maternal effects could be the cause of this difference. Plant regeneration rate was also different in the two crosses, 1.7% in direct and 2.5% in reciprocal cross.

Percent seed set between wheat and alien species, including that between tetraploïd wheat and *A. geniculata*, appears to vary and the differences are not consistent over genotypes, direction of cross, years, locations and research programs (Sharma, 1995). In the present study, seed set was low but within the range reported for this wide cross (Sharma, 1995). It varied from 3.9% the first year to 7.3% the third year (tab. 2). Also, up to 17% of hybrids produced could be transferred successfully to the field during the third year compared to 0% the first year and 10% the second year. This improvement in hybrid production was probably due to the addition of a new step for plantlet hardening during the first days following the transfer to the soil. However, the number of hybrids produced was very low and didn't improve from year to year despite our better understanding of the technical

Traits	*T. durum* parent	*A. geniculata* parent	H1 hybrid	H2 hybrid
Plant height (cm)	53	13	50	29
Number of spikes	2-8	63-82	32	23
Number of florets	12	2.6	7.4	3.8
Spike length (cm)	12.4	3.2	5.7	5.2
Leaf length (cm)	17.5	5.1	9.1	4.9
Leaf width (cm)	1.1	0.5	0.8	0.4
Pollen fertility (%)	85	80	25	3
Leaf hairiness	light hair	hairy in top leaves	very light hair	no hair
Anther dehiscence	+	+	+	-
Angled stem	no	yes	yes	yes
Resistance to leaf diseases	S	R	R	R
Plant shape	dressed	rosette	dressed	rosette

Table 3: Comparison between hybrids and their parents.

Hybrid	No chr	Genome	No. cell	No. I	II rod	II ring	II tot.	No. III	No. IV
H1	28	ABC^{u}M^o	52	16.84	4.86	0.32	5.18	0.17	0.019
H2	28	ABC^{u}M^o	28	22.90	1.5	0.06	1.56	0.65	0.00

Table 4: Average chromosome paring in H1 and H2 hybrids.

factors that limit interspecific hybridization (tab. 2). Besides the fact that some seeds were without embryos, most hybrids were weak *in vitro*. After the hybrids were successfully transferred to the pots, they were treated with colchicine but died few days later. Colchicine treatment is one cause that contributed to hybrid loss (Essad & Cachon, 1965; Lange & Jochemsen, 1992). Since only 50% of the plants were treated with colchicine solution the other half was saved.

Two hybrids were transferred to the field the second year; one produced through *T. durum* cv. Cocorit x *A. geniculata* acc. 400150 (H1) and the other from a reciprocal cross *A. geniculata* acc. 400150 x *T. durum* cv. Tensift (H2). Hybrid plants and their spikes were intermediate between the two parents (tab. 3). However, plant shape varied and was erect like durum wheat in hybrid H1 and spreading like *A. geniculata* in hybrid H2. Both hybrids inherited some traits from their alien parents such as the resistance to leaf diseases (rust and powdery mildew) and angled straw. However, regarding all the aspects together, H1 hybrid resembled more to the wheat parent and H2 to the alien parent. Reciprocal hybrids are generally close in shape to the alien parent. Plant shape, spike morphology, plant height were the traits influenced by maternal effect (tab. 3). Pollen fertility was very low in both hybrids but greater in H2.

Mitotic analysis showed that chromosome number was equal to that expected: 2n = 28; 14 chromosomes from wheat parent and the other 14 from *Aegilops* parent. Meiotic analysis showed that chromosome pairing was relatively low, especially in H2. An average of 5.18 bivalent in the H1 hybrid and 1.56 in H2 were observed. Some trivalents and tetravalents were observed but they have low frequency (tab. 4). Univalents were the most frequent reaching 22.9 in H2 hybrid. In Chinese Spring x *A. geniculata* F1 hybrid, Farooq *et al.* (1990) also noticed low pairing: up to 32.6 univalents were present in each cell. Bailey *et al.* (1993) obtained the same range (32.7) in H186 bread wheat line x *A. geniculata* cross. However, they observed no univalents when they used the ph mutant stock of Chinese Spring wheat in their cross. Low chromosome pairing confirmed differences in chromosome structure between the parental genomes (Rieger *et al.*, 1968). Ph gene in wheat which controls chromosome pairing between homoeologues was probably more active in H2 hybrid where univalent chromosomes are more frequent. *Aegilops geniculata* accession used did not inhibit this gene as do some *Aegilops* species such as *A. longissima*, *A. mutica*, *A. speltoides* (Chueca *et al.*, 1977). The low pairing frequency was also confirmed through the presence of laggard chromosomes at anaphase I (tab. 5). Over 60% pollen mother cells of the H2 hybrid showed at

Nbr laggards	0	1	2	3	4	5
H1 (*T. durum x A. genucalata*)	93.2	2.7	1.6	0.3	0.2	1.9
H2 (*A. genucalata x T. durum*)	39.6	3.1	6.4	17.1	8.8	24.9

Table 5: Percentage of laggard chromosomes in pollen mother cell of hybrids.

	H1 (*T. durum x A. genucalata*) selfed	H1 (*T. durum x A. genucalata*) backcrossed	H2 (*A. genucalata x T. durum*) selfed	H2 (*A. genucalata x T. durum*) backcrossed
Seed set (%)	2.6	10	0	3.3
Embryo rescued	-	75	-	0
Seed formed without rescue	0	38	-	0

Table 6: Seed set and germination rate in H1 and H2 hybrids.

least one laggard, and 24% had over 5 chromosomes left behind (tab. 5). In H1, where there were fewer univalents, fewer laggards were observed. These differences in chromosome behavior could be related to the direction of the cross and to the cultivar used as the female parent (Snape *et al.*, 1979).

On selfing and backcrossing, H1 hybrid produced few seeds, while H2 hybrid formed seeds only when backcrossed (tab. 6). High sterility observed in both hybrids could be explained by meiotic chromosome abnormalities which were more noticeable in H2 hybrid than H1. It could be also linked to a negative nucleo-cytoplasmic interaction as observed by Maan (1983), and Li and Dong (1991) in wheat x *Agropyron* reciprocal crosses.

Seeds produced on hybrid plants were used in successive generations. Germination rate in H1 hybrid progeny was better but only in backcross seeds. Young seedlings were planted in the field and were scored for morphological traits. Successive progenies are going to be screened for Hessian fly resistance.

Sterility in hybrids and their progeny confirmed interspecific barrier effect in *T. durum x A. geniculata* cross. Reciprocal crosses that were carried out produced more seeds and plants than direct cross. Phenotypic and cytogenetic variations observed between the two hybrids reflect maternal effect and also chromosome abnormality and less probably somaclonal variation that could have been regenerated *in vitro* where the hybrids were growing (Chen *et al.*, 1990). Additionally, the variation between the hybrid plants could also be due to heterozygosity in the wild species used or due to rapid genome changes (Sharma *et al.*, 1989). Meiotic analyses showed low chromosome pairing in the hybrids. Gene transfer by recombination will probably be limited because of lack of chromosome pairing due to the difference between the genomes.

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